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DISSOLVED SOLIDS AS HD BIOEFFLUENT TOXICANTS

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The U.S. Army, through the Alternative Technology Program at Aberdeen Proving Ground, MD, has developed a neutralization/biodegradation process that treats hydrolyzed sulfur mustard. The effluent produced from this process has been proposed to be discharged to a Federally Owned Treatment Works. Chronic toxicity studies have shown that the effluent from the bioreactors is toxic to ceriodaphnia and fathead minnows. The effluent contains 2.4% salt, which is not tolerated by freshwater organisms. The purpose of this study was to determine if the salt in the effluent was the major contributor to the toxicity. Acute toxicity studies were conducted using ceriodaphnia exposed to a mixture of bioeffluent and synthetic effluent. Results have shown that the salts were the major cause of bioeffluent toxicity.

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PREFACE

The work described in this report was authorized under Sales Order No. 8J1W5A, Alternative Technology Program. This work was started in April 1998 and completed in July 1998.

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DISSOLVED SOLIDS AS HD BIOEFFLUENT TOXICANTS

1. INTRODUCTION

The U.S. Army, through the Alternative Technology Program at Aberdeen Proving Ground (APG), MD, has developed a neutralization/biodegradation process that treats hydrolyzed sulfur mustard. Hydrolyzed mustard was biodegraded in sequencing batch reactors (SBR) that reduced the organic carbon 90-95%.[1] The effluent produced from the bioreactors was diluted with the feed stream to the Federally Owned Treatment Works (FOTW) and passed through trickling filters that represented a sewage treatment facility. The effluent produced from the trickling filters was nontoxic to ceriodaphnia and fathead minnows.[2]

The effluent produced from the SBR contained salt concentrations of 2.4%. Most of the salt in the effluent was due to the addition of sodium hydroxide needed to adjust the pH caused by the production of hydrochloric acid during the HD hydrolysis process.[3]

The effluent produced by the SBR, by definition from the Maryland Department of the Environment (MDE), is acutely toxic to ceriodaphnia. The ceriodaphnia 48-hr EC_{50} was <100% SBR bioeffluent by volume.[4] This was expected because fresh water organisms cannot tolerate high salt concentrations. Salt toxicity for *daphnia magna* ranges from 0.6-1%.[5,6] Since the ceriodaphnia are typically more sensitive than *daphnia magna*, their tolerance to salt would be less. Open literature has shown that the sensitivity to sodium chloride between *daphnia magna* and *ceriodaphnia dubia* differ by approximately 50% (ceriodaphnia being more sensitive).[7,8] Chronic toxicity tests were conducted using marine organisms (mysid shrimp and sheepshead minnows) to rule out the effects of salt in the effluent. However, the SBR effluent was toxic to these organisms due to the high ammonia levels (20-25 ppm). Typically, salt water organisms are more sensitive to ammonia than fresh water organisms.[9] The SBR feed stream was adjusted to reduce the amount of ammonia output (<5 ppm). With low ammonia levels in the effluent, it remained toxic to the marine organisms.* In reducing the nitrogen input, the SBR failed to biodegrade the organics as usual and caused the effluent to remain toxic.

Not only do high salt concentrations influence toxicity, but the ratio of the dissolved solids (salts) can also influence toxicity. The ratio of dissolved solids can interfere with transport mechanisms that can cause internal osmotic imbalances and lead to toxicity.[10,11] The toxicity to fresh water organisms can change as much as 60% when the calcium/sodium ratios are varied.[12]

The question still remains about whether the toxicity of the SBR effluent was caused by either the animals' inability to osmoregulate in a high salt environment or by residual organics that were not totally biodegradable.

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*Haley, M.V., and Kurnas, C.W., The Toxicity of HD Bioeffluents to Mysid Shrimp and Sheepshead Minnows, Research and Technology Directorate, U.S. Army Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD, December 1998, unpublished data.

Typically, when an effluent is toxic, a Toxicity Reduction Evaluation (TRE) is conducted.[13] Using various techniques such as aeration, particulate filtration, pH adjustments, C18 solid phase extraction, anion and cation exchange resin to fractionate the effluent, the cause of toxicity is isolated.[11] In some instances, the effluent becomes more toxic after treatment with exchange resins due to the addition of salts during the exchange process.

The research presented in this report was conducted in an attempt to determine if the dissolved solids in the SBR effluent caused toxicity to ceriodaphnia. The experimental design was based on a study by McCulloch,[14] who conducted a case study testing the effluent produced from a chemical facility and a steel facility. The effluents from both facilities were subjected to a TRE that could not identify the toxic component. McCulloch then prepared a synthetic effluent based on the ionic analysis of the whole effluents (no organics included). The facility effluent was diluted by the synthetic effluent and tested for toxicity using ceriodaphnia. If the organics were causing toxicity, the toxicity would decrease as the percentage of synthetic effluent increased. If the dissolved solids were causing toxicity, the toxicity would remain the same as the percentage of synthetic effluent increased.

In McCulloch's study, the concentrations were separated by a factor of 0.5 with the inclusion of 75% treatment group (12.5, 25, 50, 75, and 100%). In most of the testing conducted, there was no partial mortality. The test concentrations were set too far apart to detect slight changes in toxicity. Therefore, the calculated EC_{50} values did not change between tests, and the confidence values are suspect. In the study presented in this report, the test concentrations were arranged to provide partial mortality and yield more reliable confidence intervals (20, 15, 10, 5, and 2.5%). Past experience has shown that the salt tolerance range of ceriodaphnia was very narrow. Having a tight concentration gradient will help show small differences in toxicity and provide a more sensitive test.

Additional studies were conducted using carbon treatment to determine if the reduction of organics in the SBR effluent would reduce the toxicity. If dissolved organics could be removed and the salt concentration remain the same, then results would add more insight to the cause of SBR effluent toxicity.

2. METHODS

2.1 Synthetic Effluent Preparation.

Effluent from the SBR was analyzed for cations and anions by the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM), APG, MD (Table 1). Based on these results, a synthetic effluent was prepared using distilled water and analytical grade reagents. Table 2 lists the reagents and amounts used to prepare 1 L of synthetic effluent. Additional sodium was needed to yield the proper concentration without over shooting the anion concentrations. However, the concentration of the anions would be elevated to unacceptable levels and might cause additional toxicity. Therefore, $NaHCO_3$ was used with the assumption that HCO_3^- would be the least toxic of all the anions (toxicity testing was not conducted to confirm the assumption). This is why the concentration of HCO_3^- in the synthetic

effluent was much higher than the SBR bioeffluent. After the reagents were completely dissolved, conductivity and salinity measurements were taken, and samples were sent for analysis.

Table 1. Ionic Analysis of Dissolved Solids in SBR Effluent and Synthetic Effluent

	SBR Effluent	Synthetic Effluent
CATIONS		
Calcium (mg/L)	14.0	11.0
Magnesium (mg/L)	1.5	0.59
Potassium (mg/L)	18.0	15.0
Sodium (mg/L)	9,400.0	7,700.0
ANIONS		
Chloride (mg/L)	7,600.0	7,600.0
Fluoride (mg/L)	<5.0	<10.0
Phosphate, ortho (mg P/L)	3.4	1.1
Nitrate (mg N/L)	0.98	0.31
Nitrite (mg N/L)	<2,000.0	<50.0
Sulfate (mg/L)	6,500.0	6,200.0
Bicarbonate (mg $CaCO_3$/L)	340.0	2,420.0
Ammonia (mg/L)	8.3	5.6
Conductivity (µOMHOS)	30,600.0	30,700.0
Salinity (%)	2.4	2.3
pH	7.8	8.6

Table 2. Reagents Used to Prepare Synthetic Effluent*

	(mg)
$MgCl_2$	5.8
NH_4Cl	24.6
KCl	34.7
NaH_2PO_4	4.2
$NaNO_3$	1.3
$CaCl_2$	51.4
$NaSO_4$	9,610.0
NaCl	43,153.0
$NaHCO_3$	4,930.0

*1 L prepared

2.2 Carbon Treatment.

Samples of SBR effluent (100 mL) were placed into a beaker containing 15 g of activated carbon (20-40 mesh size). The samples were stirred for 1.5 hr, then filtered through a 0.45-μm filter. Conductivity and salinity were measured to determine any change. The reductions in dissolved organics were determined using the Chemical Oxygen Demand (COD) analysis using a colorimetric method by the HACH Company (Loveland, CO). Samples were placed into a premeasured digestion solution and heated for 2 hr at 120 °C. After the samples had cooled, absorbency was determined using a DR/2000 Direct Reading Spectrophotometer (HACH Company, Loveland, CO) set at a wave length of 620 nm. Municipal water was used during the HD hydrolysis process. Therefore, COD blanks were prepared using municipal water. Samples before carbon filtration were also subjected to COD studies to determine the amount of COD reduction due to carbon filtration.

2.3 Ceriodaphnia Acute Assays.

The *Ceriodaphnia dubia* were obtained from the Philadelphia Academy of Natural Sciences (Philadelphia, PA). The organisms were maintained as batch cultures in 800 mL of media. The batch cultures were maintained for 14 days while initiating new cultures every 5-7 days. Ceriodaphnia were grown in media consisting of well water and fed a mixture of algae and cerophyl.

Water was drawn from a 375-ft deep well and passed through a water treatment system. The treatment system consisted of air injection via a venturi tee micronizer, limestone pH adjustment, Zeta Sol Iron removal bed, carbon bed filtration, and particulate filtration. The water was stored in a darkened cabinet to prevent algae growth.

Cultures were fed a mixture of algae (*Selenastrum capricornutum*, *Chlamydomonas reinhardtii*) and cerophyl. The algae were grown in U.S. Environmental Protection Agency (EPA) media[15] for approximately 7 days before being harvested and then fed to the ceriodaphnia at a concentration of 10^6 cells/mL. The cerophyl was prepared by suspending 7.5 g of cerophyl in 1 L of distilled water. The mixture was placed on a stirring plate and stirred overnight. The cerophyl solution was filtered through three layers of cheese cloth to remove large particles. Cerophyl stock was added to the media at a concentration of 1 mL/100 mL of media.

Approximately 2 weeks before testing, 25 adults were isolated from the batch cultures for offspring production. The second brood produced was grown to adults for producing offspring (either F_3 generation or higher <24 hr old) used in testing.

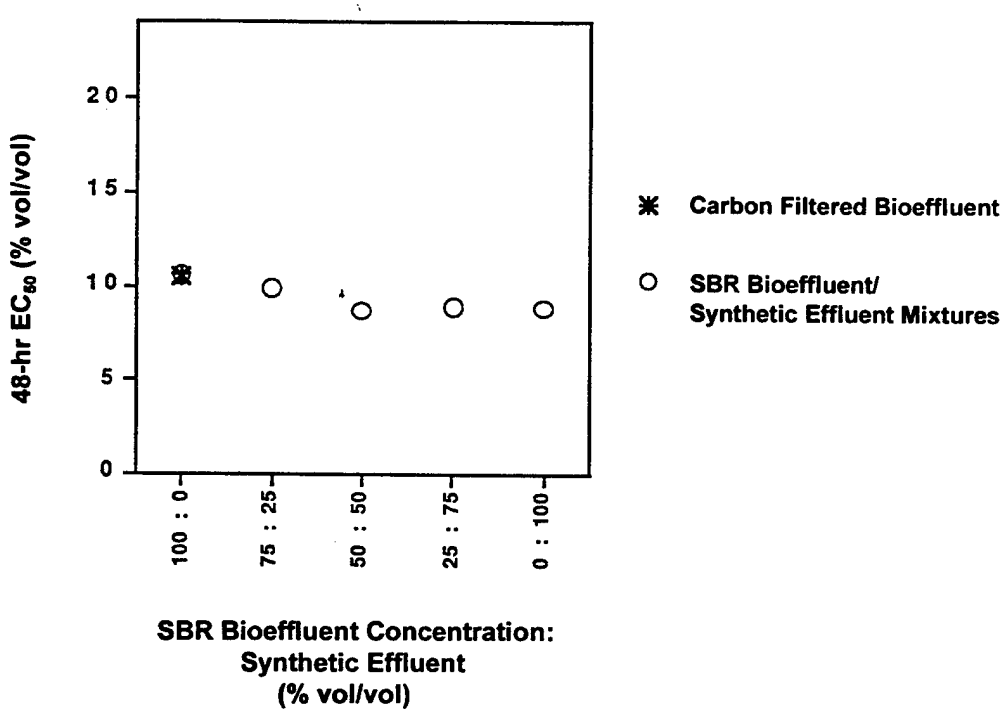
All glassware used for testing and culturing was washed with nonphosphate soap, rinsed with tap water until sudsing had ceased, rinsed twice with distilled water, then filled with distilled water and allowed to leach over night to remove any possible contaminants remaining in the glass.

The test chambers consisted of 30-mL glass beakers using a total of 15 mL of solution. There were two replicates for each treatment group (20, 15, 10, 5, and 2.5%) and

control containing 10 ceriodaphnia each. The light cycle was maintained at 16 hr light/8 hr dark. The light intensity was approximately 90 ft-c. The room temperature was maintained at 25 °C. The animals were examined under a dissecting microscope to determine mortality at 24 and 48 hr. The EC_{50} determinations were computed using the Spearman-Karber method.[16]

3. RESULTS

The toxicity of synthetic effluent was slightly higher to ceriodaphnia than the SBR bioeffluent. As the concentration of synthetic effluent increased, the toxicity increased slightly (Figure). This could possibly be due to the elevated HCO_3^-. However, the 95% confidence intervals overlapped. Therefore, the difference in toxicity was not significant (Table 3).



Note: The toxicity of SBR bioeffluent did not change when diluted with synthetic effluent. The carbon-treated SBR bioeffluent toxicity also remained the same.

Figure. Toxicity of SBR Bioeffluent/Synthetic Effluent Mixtures

When SBR bioeffluent was treated with activated carbon, the odor was eliminated, and the COD was reduced from 4,329 to 90.6 ppm (Table 4). The conductivity, salinity, and pH remained the same. The toxicity of the carbon-treated SBR bioeffluent remained the same as the untreated bioeffluent (Table 5).

Table 3. Toxicity Results of SBR Bioeffluent/Synthetic Effluent Mixtures

Concentration (% vol/vol)	100% SBR Effluent	75% SBR Effluent 25% Synthetic Effluent	50% SBR Effluent 50% Synthetic Effluent	25% SBR Effluent 75% Synthetic Effluent	100% Synthetic Effluent
	% mortality				
Control	0	0	0	0	0
2.5	0	0	0	0	0
5.0	5	5	10	15	10
10.0	35	50	70	60	65
15.0	100	100	100	100	100
20.0	100	100	100	100	100
48-hr EC_{50}% (95% Confidence Intervals)	10.5 (9.4 - 11.6)	9.8 (8.6 - 10.9)	8.6 (7.5 - 9.7)	8.9 (7.7 - 10.1)	8.8 (7.7 - 10.0)

Table 4. Water Parameter Measurements Before and After Activated Carbon Treatment

	Before Carbon Treatment	After Carbon Treatment
COD (ppm)	4,329	90.6
Conductivity (µOMHO)	30,800	30,700
Salinity (ppt)	24	24
pH	8.2	8.2

Table 5. Toxicity Results of Carbon-Treated SBR Effluent to *Ceriodaphnia dubia*

Concentration (% vol/vol)	% Mortality
Control	0
2.5	0
5.0	0
10.0	35
15.0	100
20.0	100
48-hr EC_{50}%	10.5
(95% Confidence Intervals)	(9.7 - 11.8)

Preliminary studies were conducted using Forty Fathoms (Marine Enterprises, Baltimore, MD) sea water mix to dilute outdated samples of SBR bioeffluent (sample taken 11-19-95). Acute toxicity studies were conducted on a mixture of 50% SBR bioeffluent and 50% Forty Fathoms sea water mix (salinity set at 22 ppt). The 48-hr EC_{50} results for the 50/50 mixture was 13.5% by volume (with 95% confidence intervals 10.9 - 16.0) and for 100% bioeffluent 12.3% volume (with 95% confidence intervals 10.1 - 14.3). Diluting the bioeffluent with a sea water did not significantly reduce the toxicity to ceriodaphnia.

4. DISCUSSION

If the toxicity of the SBR bioeffluent was caused by dissolved solids (salt), diluting with synthetic effluent containing similar ionic make up (no organics) will not change

the toxicity. If dissolved organics were causing the toxicity, when diluted with synthetic effluent, the toxicity would be reduced. The results of this study have shown that when the SBR bioeffluent was diluted with synthetic effluent, the toxicity to ceriodaphnia remained the same, thus indicating that dissolved solids were the cause of toxicity.

When the effluent was treated with activated carbon, the COD was reduced approximately 2 orders of magnitude. If the organics were causing toxicity, when they are removed, the toxicity should decrease. These studies have shown no change in toxicity, another indication that dissolved solids were causing the toxicity.

There were no attempts to create a synthetic effluent based solely on organic composition. Some of the identified compounds were not commercially available, and the synthesis was cost prohibited. Also, there were several compounds in very low concentrations that could not be identified. It would be virtually impossible to reproduce a synthetic media based on organic analysis.

Studies using the TRE were not conducted based on results published by McCulloch, showing that the TRE procedures have difficulty isolating toxicity when caused by dissolved solids. In many cases, the TRE procedure will add ions to the sample, causing the sample to become more toxic.

Whole Effluent Toxicity testing lacked the ability to determine if dissolved solids caused the toxicity. A survey of industrial researchers presented by Dorn[17] were in agreement on three issues: (1) salinity should not be considered a toxicant, (2) recommendations are needed when false positive results are caused by dissolved solids, and (3) if surrogate organisms (marine species) are used in an attempt to eliminate salt influence, toxicity may remain due to ionic imbalance.

5. CONCLUSION

The results presented in this report have shown that the toxicity associated with the sequencing batch reactor (SBR) effluent was due to the dissolved solids. The inability of freshwater organisms to osmoregulate in such high saline environments caused toxicity. Freshwater organisms are placed under significant osmotic stress in waters of high dissolved solids. Without protective devices, these animals either dehydrate from water loss or gain enough salt that their internal fluids cannot support the normal functions of their bodies.

Since the SBR effluent will be mixed with the waste stream preceding the Federally Owned Treatment Works (FOTW), the effluent toxicity should not be considered a problem. Precautions should be taken to guarantee the dilution levels of the SBR effluent before discharging it to the FOTW.

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